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EVALUATION OF BORAX FOR PREVENTION OF ANNOSUS ROOT ROT IN CALIFORNIA

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Abstract

Borax was effective in preventing Fomes annosus colonization of ponderosa and Jeffrey pine stumps in California. Less than one percent of the borax-treated stumps became colonized four weeks after artificial inoculation with F. annosus. Over 61 per cent of the untreated control stumps similarly inoculated and examined became colonized.

INTRODUCTION

Root rot caused by Fomes annosus is considered to be a major forest disease problem in California (3, 4, 5, 12, 13). Forest pest detection reports from many California areas indicate that this disease is becoming more widespread each year. Eastside ponderosa and Jeffrey pine forests are among the areas where it is most prevalent. Most infection centers originate through the colonization of freshly-cut green stumps (5, 7, 10).

Once established, this fungus can colonize the stump and its roots and spread via root contact to nearby healthy trees. Pines of all ages and young trees of other coniferous species are killed, sometimes within three to five years after becoming infected. Roots of mature trees of some species (particularly the true firs and incense-cedar in California) are often weakened sufficiently so that the tree is windthrown prior to being actually killed by the pathogen.

In other areas of the United States and elsewhere, the chemical treatment of freshly-cut stumps has proven an effective method of preventing stump colonization by F. annosus. Dry sodium tetraborate decahydrate (borax) appears to be the most effective and useable of the chemicals tested, with 80 to 100 percent success reported (2, 6, 8, 9, 10).

Several previous attempts to test the effectiveness of borax under California conditions have been made but were not successful, due mainly to difficulties in successfully artificially inoculating fresh-cut stumps. This test was established because of the probable future need to prevent F. annosus buildup in certain California forest areas.

OBJECTIVE

The specific objective of this study was to determine the effectiveness of dry borax treatments on freshly-cut ponderosa and Jeffrey pine as a Fomes annosus prevention measure. Because potential damage from this fungus appears to be more serious in young eastside California stands being precommercially thinned after an overstory removal cut, the study objective was further limited to these conditions. Application dates were selected to include the two seasonal periods (spring and fall) thought to be most favorable for the spread of F. annosus in California.

METHODS

Twenty-eight plots in six blocks were located on the Hat Creek District, Lassen National Forest in a randomized complete-block design.1/ All blocks selected were as uniform as possible in stocking, tree size, topography, soil and aspect, and were all in the same general vicinity. Each block was at least one airline-mile from all other areas. Blocks were not located on south slopes, dry ridge tops or exposed sites where the microclimate appeared unfavorable for F. annosus development. No annosus root rot disease symptoms were present within the blocks selected.

Three blocks were treated in the spring (May 20-23, 1969) and three in the fall (October 21-23, 1969).

Four treatments were applied to each block as follows:

A - Sprinkle stumps with dry borax and then inoculate with F. annosus.

B - Sprinkle stumps with dry borax only.

C - Inoculate with F. annosus only.

D - Control - no stump treatment.

Because of the current recommendation to use sodium nitrite for preventing stump colonization by F. annosus in eastern Canada, two additional plots were added to two of the blocks treated in the fall as follows (1, 11):

E - Spray all stumps with a 10 percent sodium nitrite solution and then inoculate with F. annosus.

F - Spray all stumps with a 10 percent sodium nitrite solution only.

These are not included in the borax treatment effectiveness analysis of variance. The particular treatment applied to a plot was determined by using a random numbers table. Plot size varied depending on stocking but most were between one-fourth and one-half acre. At least 50 leave trees and 50 stumps between two and six inches in diameter were marked with metal tags for evaluation in each plot.

1/ Steel and Torrie, 1969. Principles and Procedures of Statistics, McGraw-Hill.

Most plots had many more stumps. Only stumps between eight inches and sixteen inches in height were selected for evaluation. A one-chain buffer strip was treated in the same manner as the plot around each plot in cases where plot boundaries were this close.

In order to be certain that treated stumps could be distinguished from untreated stumps a safranine O dye was mixed with the borax (one-half teaspoon of dye per quart). Only a very light amount of borax was applied to each stump -- just enough to lightly cover the stump surface. The borax used was quite lumpy and it was rather difficult to get the dye thoroughly mixed into it. It appeared that the dye was not necessary as a few extra stumps treated with borax alone without dye were quite readily distinguishable from untreated stumps for at least ten days after treatment.

An average of 150 two-inch stumps were treated with each pound of borax. The borax used cost about 12 cents per pound. A shaker made by punching 22 holes in the lid of the pint fruit jar with an 8-penny nail was used to apply the borax. One man treating stumps right behind the sawyer could just keep up with him. Thus, the labor cost of treating was about equal to that of the thinning.

The sodium nitrite was prepared as an approximate 10 percent solution (one pint jar of sodium nitrite in one gallon of water and one teaspoon of Rhodamine B Extra dye). It was applied to the point of runoff to all cut surfaces and splintered edges with a two gallon, garden-type pressure sprayer. One man using this system could keep up with two sawyers. It was easier with the spray wand to reach through and around the thinning slash to treat the stumps than it was with the borax shaker jar. The sodium nitrite used cost $.96 per pound or $.96 per gallon of final solution. One gallon of solution treated about 500 two-inch stumps. The cost of the dye, which was easy to get into solution and a very brilliant red that showed up very well on the treated stumps, was negligible.

All inoculum was made up in the field just prior to inoculating. Conidia for this was grown in standard petri plates for three to four weeks prior to inoculation. Each batch of inoculum was made by washing and scraping ten plates with abundant sporulating conidiophores into 32 ounces of distilled water.

All inoculations were made with a one-quart plastic sprayer. Each stump inoculated was uniformily covered with a fairly heavy mist of inoculum. About 10 ounces of inoculum was used in each plot (50 stumps). A kraft paper bag sunshade was placed loosely over each inoculated stump and tacked down with a stapler immediately after inoculation.

Six to ten, thin (about 3/8") fresh-cut ponderosa pine disks were placed on wet cotton in petri plates and inoculated with the same inoculum after each series of stumps was inoculated. These were stored in the laboratory at room temperatures for about six weeks and then examined under a stereoscopic microscope for Oedocephalum conidiophores. See Table I.

Six spore traps made in a similar manner were set out in most plots immediately after treatment. These plates were left open and collected after about 24 hours exposure. F. annosus colonized on only two out of the 72 disks exposed during the spring treatment period but during the fall treatment period it colonized on 42 out of 64 disks exposed, indicating that the fall period days were very favorable for natural F. annosus infection.

RESULTS

Four weeks after treatment, three consecutive disks (each about two inches thick) were cut from the top of each of 25 tagged stumps (collected at random) from each plot. Twelve weeks after treatment similar disks were cut from the remaining 25 tagged stumps in each plot.

Each disk was dipped momentarily in a five percent solution of sodium hypochlorite, then rinsed in water, then dipped into 95 percent ethanol, and then drained about two minutes before wrapping in newspaper.

All disks collected from the blocks treated in the spring and those from the first 25 stumps from each plot treated in the fall were then wrapped in newspaper, dipped into water and then packed into metal garbage cans for transportation to the laboratory. The last set of disks collected from the fall-treated plots were treated the same way except that they were packaged in plastic bags instead of newspaper. No water was added to these packages. Each disk was numbered with a felt tip marker so that the plot number and its position on the stump could be determined in the laboratory.

All disks were split into four sections in the laboratory within four days of field cutting. A small chip was taken from each split face and plated into a F. annosus selective agar. 2/ All four chips from a disk were surface sterilized in 95 percent ethanol and then plated into the same petri dish. The disks were then rewrapped in the newspaper and packed back into the garbage cans.

2/ Kuhlman, E.G., and Hendrix, F.F., 1962. Phytopathology 52: 1310-1312.

TABLE I

Artificial Inoculum Checks

	Number Of Disks	Percent With Annosus
Inoculated - 5/20-21, 1969 Examined - 6/6 and 6/19, 1969		
Area #1		
Plot A	10	80
Plot C	10	100
Area #2		
Plot A	10	100
Plot C	10	100
Area #3		
Plot A	10	100
Plot C	10	100
Inoculated - 10/21-23, 1969 Examined - 10/31 and 11/15, 1969		
Area #4		
Plot A	5	100
Plot C	5	100
Area #5		
Plot A	5	100
Plot C	5	100
Plot E	10	20
Area #6		
Plot A	5	100
Plot C	5	100
Plot E	10	90

	1/ Collection Delay	Stumps With Annosus	Total Stumps	Percent With Annosus	Arcsin Transformation
Plots Treated 5/20-23, 1969					
Area #1					
Plot A	4	0	24	0	0
	12	0	25	0	0
	T	0	49	0	0
B	4	0	25	0	0
	12	0	25	0	0
	T	0	50	0	0
C	4	21	24	87.5	69.30
	12	0	24	0	0
	T	21	48	43.8	41.44
D	4	3	25	12.0	20.27
	12	0	25	0	0
	T	3	50	6.0	14.18
Area #2					
Plot A	4	0	25	0	0
	12	0	25	0	0
	T	0	50	0	0
B	4	0	24	0	0
	12	0	25	0	0
	T	0	49	0	0
C	4	18	24	75.0	60.00
	12	0	25	0	0
	T	18	49	36.7	37.29
D	4	1	24	4.2	11.83
	12	0	25	0	0
	T	1	49	2.0	8.13
Area #3					
Plot A	4	1	24	4.2	11.83
	12	1	25	4.0	11.54
	T	2	49	4.1	11.68
B	4	0	25	0	0
	12	0	25	0	0
	T	0	50	0	0
C	4	14	25	56.0	48.45
	12	2	25	8.0	16.43
	T	16	50	32.0	34.45
D	4	0	25	0	0
	12	0	25	0	0
	T	0	50	0	0

1/ Elapsed time in weeks between treatment and collection of stump samples.

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Of All Data

	Collection Delay 1/	Stumps With Annosus	Total Stumps	Percent With Annosus	Arcsin Transformation
Plots Treated 10/21-23, 1969					
Area #4					
Plot A	4	0	25	0	0
	12	0	25	0	0
	T	0	50	0	0
B	4	0	25	0	0
	12	0	24	0	0
	T	0	49	0	0
C	4	19	25	76.0	60.67
	12	15	25	60.0	50.77
	T	34	50	68.0	55.55
D	4	0	25	0	0
	12	1	25	4.0	11.54
	T	1	50	2.0	8.13
Area #5					
Plot A	4	0	25	0	0
	12	0	24	0	0
	T	0	49	0	0
B	4	0	25	0	0
	12	0	24	0	0
	T	0	49	0	0
C	4	13	25	52.0	46.15
	12	19	25	76.0	60.67
	T	32	50	64.0	53.13
D	4	0	25	0	0
	12	1	25	4.0	11.54
	T	1	50	2.0	8.13
E	4	0	25	0	-
	12	0	25	0	-
F	4	0	25	0	-
	12	0	25	0	-
Area #6					
Plot A	4	0	24	0	0
	12	0	25	0	0
	T	0	49	0	0
B	4	0	25	0	0
	12	0	25	0	0
	T	0	50	0	0
C	4	5	25	20.0	26.56
	12	14	24	58.3	49.78
	T	0	49	38.8	38.53
D	4	0	23	0	0
	12	2	25	8.0	16.43
	T	2	48	4.2	11.83
E	4	0	24	0	-
	12	0	25	8.0	-
F	4	0	25	4.2	-
	12	0	25	0	-

ANALYSIS OF VARIANCE USING ALL DATA
(arcsin transformation)

Treatments	Blocks						Treatment Totals		Treatment Means
	I	II	III	IV	V	VI	X	ΣX^2	
A	0	0	11.68	0	0	0	11.68	136.42	1.95
B	0	0	0	0	0	0	0	0	0
C	41.44	37.29	34.45	55.55	53.13	38.53	260.39	11,687.78	43.40
D	14.18	8.13	0	8.13	8.13	11.83	50.40	539.31	8.40
Block Totals X	55.62	45.42	46.13	63.68	61.26	50.36	322.47		
ΣX^2	1918.35	1456.64	1323.22	3151.90	2888.89	1624.51		12,363.51	

F RATIO COMPUTATION

Source Of Variation	df	SS	MS
Blocks	5	74.36	14.87
Treatments	3	7413.80	2471.27
Error	15	542.56	36.17
Total	23	8030.72	

$$F \text{ (treatments)} = \frac{\text{Treatment MS}}{\text{Error MS}} = \frac{2471.27}{36.17} = 68.32$$

Therefore, treatments are significant at the 5 percent level.

After about 10 days of incubation at room temperatures both the plates and the stump disks were examined with a stereoscopic microscope. The presence of Oedocephalum conidiophores in any amount on either the plates or the stump disks was considered positive evidence of successful colonization by F. annosus. See Table II.

Tukey's procedure to judge the significance of treatment differences was used as follows:

$$\text{Standard error of the mean } (S_{\bar{x}}) = \frac{36.16}{6} = 2.46$$

Tukey's W = Table Value at the 5 percent level for 4 treatments and 15 error degrees of freedom $X_{\bar{x}}$

$$W = 4.08 \times 2.46 = 10.04$$

Treatment mean array:

B	A	D	C
0	1.95	8.40	43.40

Since none of the differences between the means for Treatments A, B or D is greater than 10.04 (Tukey's W) there is no reason to believe that these treatments are significantly different from each other. Because the difference between the mean of Treatment C and all other treatment means is much greater than 10.04 it can be considered significantly different. Therefore, on the basis of artificial inoculation, borax can be considered effective in preventing stump colonization by F. annosus.

Because natural infection on the untreated-uninoculated controls was so light and variable it was masked in this total data test. An analysis of variance and computation of Tukey's W, using only the natural infection (Treatment D) and the borax alone (Treatment B) data gives a value of 4.38. Because the difference between these treatment means (8.40) is much greater than this, it can be concluded that the borax treatment is also effective in preventing natural F. annosus infection.

Leave trees will be examined as often as necessary in the future to determine cause of death for any mortality that may occur. The amount of loss due to F. annosus, if any, will be determined for each plot and analysed when appropriate -- probably after about five growing seasons from time of cutting.

DISCUSSION

Borax is effective in preventing the establishment of F. annosus colonization of fresh-cut pine stumps. An average of less than one percent of the borax-treated pine stumps became infected when artificially inoculated as compared to an average of about 60 percent of the untreated pine stumps inoculated in the same manner.

Although the use of sodium nitrite to prevent Fomes annosus colonization of fresh-cut pine stumps was not included in the original test design, the two fall treatments applied indicate that this material has promise. Under the conditions tested the sodium nitrite solution was easier to apply than the borax. However, some corrosion difficulties with the spray equipment were experienced and if used during the winter months an anti-freeze would have to be added to the solution. Also, since sodium nitrite is more toxic than borax, further testing at this time is probably not warranted.

Collecting the stump material and culturing about four weeks after treatment was the most satisfactory under the conditions tested. Waiting as much as twelve weeks after the spring treatment during a hot summer period proved too long. Very little F. annosus was was found in the untreated-inoculated stumps collected twelve weeks after the spring treatments. Because numerous other fungi developed on the stump disks collected under these conditions it is assumed that the F. annosus was either masked by or replaced by these other organisms in the portion of the stump collected and cultured. Waiting twelve weeks after the fall treatment, during cool weather including some freezing temperatures, was satisfactory but no better than four weeks.

Packaging the stump disks in plastic bags rather than in wet newspaper during the fall collection period proved much easier and quite satisfactory. Because all pine stumps have a rather high moisture content, four weeks after cutting, regardless of season, it should be possible to use this technique at all times of the year.

In almost every case when the stump material was collected four weeks after treatment the F. annosus found was in the top disk (about the first two inches) of the stump. Therefore, it appears satisfactory to sample only from this portion of the stump four weeks after treatment. As well as reducing the amount of work involved, this technique also has the added advantage of being able to conclude with considerable confidence that the F. annosus recovered came in via the stump surface and not from infected roots or other in-soil sources.

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SOME FOREST TREE DISEASES IN CALIFORNIA . . .

ACTIVE CONTROL PROGRAMS

WHITE PINE BLISTER RUST Caused by the introduced fungus, Cronartium ribicola, which attacks all white (5 needle) pines sometimes killing young trees. Control presently is by eradicating the alternate host (gooseberry and currant plants), and in some areas, by more direct means. A Statewide control program has been underway for many years. The program is now being revised.

DWARF MISTLETOE Caused by parasitic flowering plants, Arceuthobium spp., which attack all pines, firs, and Douglas-fir seriously retarding growth and sometimes killing trees. Control is by removal of infected trees during logging and thinning. Control by pruning infected branches is possible on some trees. A control program has been underway for ten years.

ROOT DISEASES

ANNOSUS Caused by the fungus, Fomes annosus, which attacks all native conifers and a few shrubs, killing pines and causing butt rot in firs. The pathogen spreads by wind-borne spores that invade freshly-cut stumps, after which the fungus grows through the roots and then by root contacts infects uninfected trees. The disease is widely distributed in California; its importance for timber and recreation management is being studied. The effectiveness of borax as a stump-treatment material is being tested.

ARMILLARIA Caused by the fungus, Armillaria mellea, which attacks many conifers and hardwoods, occurring as a parasitic killer, a heart rot, and a saprophyte of dead material. Although widely distributed in California, and a cause of serious local loss, the overall impact of Armillaria probably is not great.

NEEDLE DISEASES

ELYTRODERMA Caused by the fungus, Elytroderma deformans, which unlike other needle diseases, may be perennial in its hosts, ponderosa and Jeffrey pine. Seriously infected trees retain few needles other than those of the current season, and growth is reduced. In California, elytroderma is confined largely to small infection centers where local conditions are especially favorable.

RHABDOCLINE Caused by the fungus, Rhabdocline pseudotsuga, a needle cast disease, which occasionally causes severe needle discoloration and loss in Douglas-fir. The disease can be serious in Christmas tree plantations.

RUSTS

GALL RUST Caused by the fungi Peridermium harknessii. It commonly produces ball-like cankers on branches of two- and three-needle pines, although trunk cankers are formed as well. The fungus is prevelant throughout the State, but is of little importance, except locally; it can be severely damaging on Monterey and bishop pine in coastal areas.

LIMB RUST Caused by the fungi, Peridermium filamentosum and P. stalactiforme, which, unlike other rusts of pine, are systemic, spreading slowly from limb to limb; infected trees are weakened and sometimes killed.

HEART ROTS

The principal heart rots (saprophytes, not parasitic killers of living trees) are red ring rot, Fomes pini, and Indian paint fungus, Echinodontium tinctorium. The first is a major source of defect in pines and Douglas-fir; the second in true firs. They are important both because of the wood destroyed, and because they are causes of hazardous trees.